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<b>APPELLANTS' BRIEF</b>  Address to: Mail Stop Appeal Brief-Patents Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450	Application Number	10/716,349
	Confirmation Number	7039
	Attorney Docket No.	SEEK-001CON
	Filing Date	November 17, 2003
	First Named Inventor	Ellen L. Berg
	Examiner	Karlheinz Skowronek
	Group Art	1631
Title: <i>Function Homology Screening</i>		

Sir:

This Brief is filed in support of Appellants' appeal from the Examiner's Rejection dated September 25, 2008. No claims have been allowed. Claims 17 and 19-22 are pending and appealed herein. Claims 18, 23 and 24 have been withdrawn from consideration. A Notice of Appeal was filed on December 29, 2008. Accordingly, this Appeal Brief is timely filed.

The Board of Appeals and Interferences has jurisdiction over this appeal pursuant to 35 U.S.C. §134.

Appellants believe that no fees are due, in view of Appellants previously filed Brief of July 7, 2008 in which the fees were paid. As noted in the Office Action of December 28, 2008, the previously paid appeal brief fee can be applied to the new appeal.

In the unlikely event that the fee transmittal or other papers are separated from this document and/or other fees or relief are required, Appellants petition for such relief, including extensions of time, and authorize the Commissioner to charge any fees under 37 C.F.R. §§ 1.16, 1.17 and 1.21 which may be required by this paper, or to credit any overpayment, to deposit account number 50-0815, reference no. SEEK-001CON.

**REAL PARTY IN INTEREST**

The inventors named on this patent application assigned their entire rights to the invention to BioSeek, Inc.

**RELATED APPEALS AND INTERFERENCES**

There are currently no other appeals or interferences known to Appellants, the undersigned Appellants' representative, or the assignee to whom the inventors assigned their rights in the instant case, which would directly affect or be directly affected by, or have a bearing on the Board's decision in the instant appeal.

A Notice of Appeal has been filed in the related case, USSN 10/220,999.

**STATUS OF CLAIMS**

The present application was filed on November 17, 2003, with Claims 1-16. During the course of prosecution, Claims 17-24 were added, Claims 1-16 were canceled, and Claims 18, 23, and 24 were withdrawn by the Examiner as being directed to a non-elected invention. Accordingly, Claims 17 and 19-22 are pending in the present application, all of which stand rejected. All of the rejected claims are appealed herein.

**STATUS OF AMENDMENTS**

No amendments to the Claims were filed subsequent to issuance of the Final Rejection.

**SUMMARY OF CLAIMED SUBJECT MATTER**

The claimed invention is drawn to a method for analyzing a candidate compound for a biological activity of interest by using the measurement of multiple parameters in a cell culture assay to produce a biological dataset profile that is indicative of the pathways that are active in the cell culture.

Below is a description of each appealed claim and where support for each can be found in the specification.

Claim 17 claims a method for analyzing a candidate compound for a biological activity of interest, the method including contacting a test mammalian cell culture with the compound, in which the culture includes a plurality of factors in which a plurality of signalling pathways is induced by the presence of the factors; measuring at least two parameters associated with the plurality of pathways and comparing the measurement of the at least two parameters with the measurement from a

control cell culture lacking the compound; and recording the measurements of the test cell culture and the control cell culture to produce a biological dataset profile, in which the biological dataset profile is indicative of the pathways that are active in the cell culture (see specification at page 12, line 7 through page 13, line 24 and page 14, lines 12-30).

Claim 19 claims the method of Claim 17, in which the cells are primary cells (see specification at page 17, line 29 through page 18, lines 10).

Claim 20 claims the method of Claim 17, in which the test cell culture includes at least one activator of a pathway active in the cell culture (see specification at Figures 6 through 9; page 25, lines 1-10; page 30, lines 6-17; page 33, lines 10-22).

Claim 21 claims the method of Claim 17, in which the test cell culture includes at least one inhibitor of a pathway active in the cell culture (see specification at Figures 4 and 5; page 27, line 28 through page 28, line 5; page 32, lines 1-17; page 37, lines 1-6).

Claim 22 claims the method of Claim 17, further including the step of compiling a plurality of the biological dataset profiles in a database (see specification at page 4, line 33 through page 5, line 2; page 16, line 31 through page 17, line 2).

**GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL**

I. Claim 22 has been rejected under 35 U.S.C. 112, second paragraph as having insufficient antecedent support for the phrase "the biological dataset profiles" in line 2.

II. Claim 17 and 22 have been rejected under 35 U.S.C. 102(e) as being anticipated by Friend et al., U.S. Patent no. 6,801,859 as evidenced by Chung et al. (1982) J. Cell Biology 95:118-126.

III. Claims 17 and 19-22 have been rejected under 35 U.S.C. 103(a) as unpatentable over Friend et al., U.S. Patent no. 6,801,859 in view of Chung et al. (1982) J. Cell Biology 95:118-126.

IV. Claims 17 and 19-22 have been rejected under 35 U.S.C. 103(a) as unpatentable over Friend et al., U.S. Patent no. 6,801,859 in view of Rice et al. (1996) Analytical Biochemistry 241:254-259.

**ARGUMENT****I. Claim 22 meets the requirements of 35 U.S.C. 112, second paragraph.**

The Examiner has rejected Claim 22 as lacking insufficient antecedent support for the phrase, "the biological dataset profiles" in line 2. Appellants respectfully submit that the claim meets the requirements of 35 U.S.C. 112, second paragraph, which recites in its entirety, "the specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention".

The phrase asserted to lack antecedent support reads as follows, "further including the step of compiling a plurality of the biological dataset profiles in a database". The phrase properly references the language of the claim from which it depends, in which a method for generating a biological dataset profile is provided.

Claim 22 further includes a step not set forth in the base claim, in which a plurality a biological dataset profiles, each as set forth in the base claim, are compiled.

One of skill in the art would readily understand the reference in Claim 22 to biological dataset profiles, which are defined in the base claim, wherein, because the claim references a plurality of such datasets, they are appropriately referred to in the plural.

Appellants respectfully submit that Claim 22 is patentable under 35 U.S.C. 112, second paragraph. Reversal of the rejection is requested.

**II. Claims 17 and 22 are not anticipated under 35 U.S.C. § 102(b) by Friend *et al.*, U.S. Patent no. 6,801,859 as evidenced by Chung *et al.* (1982).**

The Examiner has rejected claims 17 and 22 as being anticipated by Friend *et al.*, U.S. Patent no. 6,801,859, as evidenced by Chung *et al.* (1982). In making this rejection, the Examiner asserts that Friend *et al.* teaches each and every element of the claims. It is asserted that it is inherent to the culture of mammalian cells to include a plurality of factors that affect a plurality of signaling pathways (Office Action of 9/25/08, page 6) as evidenced by Chung *et al.* (1982).

A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference. *Verdegaal Bros. v. Union Oil of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987).

For the reasons detailed below, Appellants submit that the cited references fail to anticipate the claimed invention. Specifically, Appellants submit that Friend *et al.* in view of Chung *et al.* fail to teach, either expressly or inherently, a cell culture including a plurality of factors in which a plurality of signaling pathways is induced by the presence of the factors, as is claimed. The references

further fail to teach measuring at least two parameters associated with the plurality of pathways and comparing the measurement of the at least two parameters with the measurement from a control cell culture lacking the compound, as is claimed.

The invention of the present claims, as set forth in independent Claim 17, recites the step of contacting a mammalian cell culture with a compound to be characterized, where the cells in the culture are activated by at least two factors. The cited reference, in contrast, describes methods for screening molecules using cell co-cultures, wherein the cell cultures differ by expression of a single target gene by the over- or under-expression of that target gene. The Friend *et al.* methods do not employ a cell culture comprising at least two factors or in which a plurality of pathways is activated.

The Examiner has reiterated that it is inherent to the culture of mammalian cells to include a plurality of factors that affect a plurality of signaling pathways as evidenced by Chung *et al.*, who demonstrate the culture of rabbit kidney cells in a culture medium. Applicants respectfully submit that it is not inherent to cell cultures to activate a plurality of pathways through multiple factors. It is possible to design a culture where a plurality of pathways is activated. Similarly it is possible to design a culture where a plurality of pathways is not activated. For example, Chung *et al.* found that the rabbit kidney cultures required a factor such as insulin, but cultures did not respond to factors such as EGF and T<sub>3</sub>.

For example, cells that are cultured continuously in the presence of a factor may down-regulate their receptors, such that over time they no longer respond to the factor, or become refractory. The concentration of factors may be insufficient to induce a plurality of pathways. Cells may also respond in an oscillatory fashion to factors in the culture medium, such that depending on the time point of assessment, the plurality of signaling pathways is not induced. In other cases, the presence of one factor may abrogate the signaling activity of another, for example retinoids suppress epidermal growth factor-associated cell proliferation by inhibiting epidermal growth factor receptor-dependent ERK1/2 activation. Applicants respectfully submit that the evidence of Chung *et al.* does not inherently provide Friend *et al.* with each and every element of the present claims, which specify that a plurality of signaling pathways is induced.

In the methods of the present invention, a test agent contacts cells in culture that are stimulated in multiple pathways by the addition of at least two factors. Appellants respectfully submit that there is no teaching by Friend *et al.* as evidenced by Chung *et al.* that would inform one of skill in the art to perform such analysis in the presence of at least two factors acting on the cell.

Appellants have observed that the activation of cells in multiple pathways reveals properties of test agents that are cryptic in the absence of these factors. Many biologically active agents were

found to have no detectable change in parameters when brought into contact with unstimulated cells, as can be found in many cell culture systems. Yet when a biologically active agent is added to cells stimulated in multiple pathways, as in the methods of the invention, distinctive parameter changes could be observed. Accordingly, Appellants submit that "a plurality of factors that affect a plurality of signaling pathways" as set forth in the claimed invention cannot be inherently equivalent to endogenous cell-culture factors, which are asserted to be invariantly present in any cell culture system, because such endogenous factors do not inherently activate a plurality of signaling pathways.

The Examiner has further stated that the addition of factors simultaneously with the agent is not recited in the rejected claims. Appellants submit that the instant claims positively recite that the "biological dataset profile is indicative of the pathways that are active in said cell culture." Upon reading the claims in light of the specification, as the law requires, it is clear to the ordinarily skilled artisan that the claimed "factors" must be as described in the specification, i.e. specific factors added to stimulate signaling pathways, and not simply endogenous factors which are always present in any cell culture. In contrast, the factors contemplated by the methods of the instant claims are modulatory of specific pathways in order for the dataset profile to be informative, as required by the claim.

The Patent Office asserts that Friend *et al.* teaches the use of mammalian cell cultures, at column 44, line 39-40 and column 10 lines 56-59, in the context of cell systems having perturbed biochemical pathways. Appellants respectfully submit that the citations fail to teach the methods of the present invention.

Col. 10, lines 56-59 of Friend *et al.* reads as follows:

In most preferred embodiments of the invention, the cells  
55 used for cluster analysis are of the same type and from the  
same species as the species of interest. For example, human  
kidney cells are preferably tested to identify consensus  
profiles to evaluate drugs or therapies that are used to treat  
disorders involving human kidney cells. However, in some  
60 preferred embodiments, the biological samples are not of the  
same type or are not from the same species as the species of  
interest. For example, in certain preferred embodiments,  
yeast cells may be used to define consensus profiles that are  
useful, e.g., in comparing or evaluating drugs or drug  
65 candidates used or intended for human therapies.

Col. 44, lines 39-40 of Friend *et al.* reads as follows:

35 For each of the mammalian expression systems described above, as is widely known to those of skill in the art, the gene of interest is put under the control of the controllable promoter, and a plasmid harboring this construct along with  
 40 an antibiotic resistance gene is transfected into cultured mammalian cells. In general, the plasmid DNA integrates into the genome, and drug resistant colonies are selected and screened for appropriate expression of the regulated gene. Alternatively, the regulated gene can be inserted into an episomal plasmid such as pCEP4 (Invitrogen, Inc.), which  
 45 contains components of the Epstein-Barr virus necessary for plasmid replication.

Appellants respectfully submit that the cited sections of Friend *et al.* do not teach a method of analysis wherein an agent is contacted with a mammalian cell culture, wherein said culture comprises a plurality of factors and wherein a plurality of pathways is induced by the factors. The cited paragraph from column 10 suggests that screening drugs for treatment of kidney cancer might use kidney cancer cells, but that in preferred embodiments, yeast cells are used. The cited paragraph from column 44 teaches a gene of interest may be expressed on a vector. It is not seen how these sections could be interpreted as teaching cell cultures in which a plurality of factors is used to activate a plurality of signaling pathways.

Appellants respectfully submit that the cited art fails to teach each and every element set forth in Claims 17 and 22, either expressly or inherently described, in a single prior art reference. Reversal of the rejection is requested.

III. Claims 17 and 19-22 are not made obvious by the combination of Friend *et al.*, U.S. Patent no. 6,801,859 in view of Chung *et al.* (1982) J. Cell Biology 95:118-126.

With respect to the rejection under 35 U.S.C. § 103(a), the Appellants will argue the rejected claims in Groups as follows:

**Group I:** Claims 17 and 22, drawn to a method for analyzing a candidate compound for a biological activity of interest, the method including contacting a test mammalian cell culture with the compound, in which the culture includes a plurality of factors in which a plurality of signaling pathways is induced by the presence of the factors, measuring at least two parameters associated with the plurality of pathways and comparing the measurement of the at least two parameters with the measurement from a control cell culture lacking the compound, and recording the measurements of the test cell culture and the control cell culture to produce a biological dataset

profile, in which the biological dataset profile is indicative of the pathways that are active in the cell culture, which results may be collected in a database as set forth in Claim 22;

**Group II:** Claim 19, drawn to the method of Claim 17, in which the cells are primary cells;

**Group III:** Claim 20, drawn to the method of Claim 17, in which the test cell culture includes at least one activator of a pathway active in the cell culture; and

**Group IV:** Claim 21, drawn to the method of Claim 17, in which the test cell culture includes at least one inhibitor of a pathway active in the cell culture.

*Group I: Claims 17 and 22*

Appellants submit that Friend *et al.* in view of Chung *et al.* fail to teach, either expressly or inherently, a cell culture including a plurality of factors in which a plurality of signaling pathways is induced by the presence of the factors, as is claimed. The references further fail to teach measuring at least two parameters associated with the plurality of pathways and comparing the measurement of the at least two parameters with the measurement from a control cell culture lacking the compound, as is claimed.

The invention of the present claims, as set forth in independent Claim 17, recites the step of contacting a mammalian cell culture with a compound to be characterized, where the cells in the culture are activated by at least two factors. The primary reference, in contrast, describes methods for screening molecules using cell co-cultures, wherein the cell cultures differ by expression of a single target gene by the over- or under-expression of that target gene. The Friend *et al.* methods do not employ a cell culture comprising at least two factors or in which a plurality of pathways is activated.

The Examiner has stated that it is inherent to the culture of mammalian cells to include a plurality of factors that affect a plurality of signaling pathways as evidenced by Chung *et al.*, who demonstrate the culture of rabbit kidney cells in a culture medium. Applicants respectfully submit that it is not inherent to cell cultures to activate a plurality of pathways through multiple factors. It is possible to design a culture where a plurality of pathways is activated. Similarly it is possible to design a culture where a plurality of pathways is not activated. For example, Chung *et al.* found that the rabbit kidney cultures required a factor such as insulin, but cultures did not respond to factors such as EGF and T<sub>3</sub>.

For example, cells that are cultured continuously in the presence of a factor may down-regulate their receptors, such that over time they no longer respond to the factor, or become refractory. The concentration of factors may be insufficient to induce a plurality of pathways. Cells may also respond in an oscillatory fashion to factors in the culture medium, such that depending on

the time point of assessment, the plurality of signaling pathways is not induced. In other cases, the presence of one factor may abrogate the signaling activity of another, for example retinoids suppress epidermal growth factor-associated cell proliferation by inhibiting epidermal growth factor receptor-dependent ERK1/2 activation. Applicants respectfully submit that the evidence of Chung *et al.* does not inherently provide Friend *et al.* with each and every element of the present claims, which specify that a plurality of signaling pathways is induced.

In the methods of the present invention, a test agent contacts cells in culture that are stimulated in multiple pathways by the addition of at least two factors. Appellants respectfully submit that there is no teaching by Friend *et al.* in view of Chung *et al.* that would inform one of skill in the art to perform such analysis in the presence of at least two factors acting on the cell.

Appellants respectfully submit that the unexpected benefits of the claimed invention are evident in the Examples provided in the present application. The experiments set forth in paragraphs 208-216; Figures 4A – 4C; and Table 1 are illustrative of the unexpected benefits of Applicants' methods, and provide a useful comparison with the cited prior art.

These experiments utilize cultures of HUVEC cells that are activated in multiple pathways with TNF- $\alpha$ ; IFN- $\gamma$ ; and IL-1 (paragraph 208). After addition of the factors and a test agent, the cells are cultured and assays for expression of the parameters: ICAM-1, VCAM-1, E-selectin, IL-8, CD31, HLA-DR and MIG (paragraph 211).

The parameter data for a large number of agents are shown in Table 1, and the clustering for mechanism of action obtained with multiparameter clustering analysis is shown in Figure 4C.

Some selected data sets from Table 1 include:

Inhibitor Class	UID	Compound	Conc.	Units	1	2	3	4	5	6	7
Antioxidant	181	N-acetylcysteine	5.00	$\mu$ M							
Corticosteroid	241	Prednisolone	160.00	$\mu$ M							
NF $\kappa$ B	4	AA861	20.00	$\mu$ M		NN	NN	ND	NN		
p38 MAPK	730	SB 203580	80.00	$\mu$ M							ND N
p42/44 MAPK	221	PD098059	18.70	$\mu$ M					ND	ND	
Tyr Kinase	733	AG126	25.00	$\mu$ M	NN						
N/A	521	Control									

Applicants wish to point out what is evident from even this small subset of Table 1 information – that no single parameter can sufficiently characterize the mechanism of action for a test agent. If one views the information provided in Table 1 as a single parameter point, next to another single parameter point, one could never determine the pathways in which a test agent is acting. This is because there is insufficient discrimination from a single parameter. It is only when

one considers multiple parameters, as set forth in the present claims, that one can distinguish the action on cellular pathways.

Applicants note that the cited prior art Friend *et al.* do not teach methods wherein a test agent is added to a cell culture in the presence of factors stimulating at least two signaling pathways. Applicants have observed that the activation of cells in multiple pathways reveals properties of test agents that are cryptic in the absence of these factors. Many biologically active agents were found to have no detectable change in parameters when brought into contact with unstimulated cells. Yet when added to cells stimulated in multiple pathways, as in the methods of the invention, distinctive parameter changes could be observed.

For example, the compounds AA861, SB 203580, PD098059, and AG126 can be added to a culture of unstimulated HUVEC cells, and after 24 hours the parameter read-outs for ICAM-1, VCAM-1, E-selectin, IL-8, CD31, HLA-DR and MIG would be indistinguishable from controls lacking the agents; and thus would be indistinguishable from each other. Yet, when applied to stimulated cells each of these agents generated a distinctive profile typical of the mechanism of action, *i.e.* NFkB signaling pathway vs. p38 MAPK pathway vs. P42/44 MAPK signaling pathway, etc.

In comparison, the Examiner's attention is drawn to the methods of Friend *et al.* Friend *et al.* teach methods that would not have suggested those of the invention to one of ordinary skill in the art at the time the present invention was made. The Friend *et al.* methods are characterized as follows:

Specifically, these methods allow screening for modulators of biomolecules, including screening for antimicrobial agents, by the use of cell types which exhibit conditional growth phenotypes. . . .

The method includes determining the effects of a potential modulator by comparing a phenotypic sensor between a first cell type having an altered biomolecule and a second cell type having a normal biomolecule. The cell type having an altered biomolecule has a conditional growth phenotype, or the altered biomolecule has a partially crippled function. . . . At least one of the first and second cell types are grown in contact with a potential modulator in an appropriate growth medium, preferably under semi-permissive conditions, such that the function of the altered biomolecule is partially crippled. Preferably the first and second cell types are separately in contact with the potential modulator. . . .

A "modulator" or 'modulator of a biomolecule" or "biomodulator" is an agent which is able to affect the activity of a biomolecule by either inhibiting or enhancing that activity. Generally, such a modulator is an inhibitor of the biomolecule. Thus, modulators include, for example, antimicrobial agents and anticancer agents. A "known biomodulator" is a compound which is known to be biologically active on particular cell, but the particular mode of action or cellular target need not be known. A "potential modulator" is a test compound is a screen or evaluation.

The methods taught by Friend *et al.* differ from those of the present invention in important ways that highlight important aspects of the invention. Friend *et al.* methods do **not**:

- Stimulate specific multiple pathways
- compare results against activated cultures in the absence of the agent.
- Involve determining pathways of action. Friend *et al.* methods do not involve the performance of such an analysis.

The present claims are drawn to various methods for generating and utilizing a dataset of parameter values obtained from cells under specific culture conditions – termed a “biomap” or a “biomap profile” – in determining the effect of an agent on a cellular signaling pathway. The subject methods provide robust results having enhanced predictability in relation to a physiological state of interest, by providing for the culture systems where multiple pathways are induced, and where multiple parameters are measured and compared to control assay combinations.

Applicants’ invention provides methods that harness cellular complexity to provide insight into the pathways affected by a genetic agent of interest. Such analysis provides a much deeper understanding of gene action than can be readily obtained by methods taught by the prior art, and such a deeper understanding is very useful in drug development, elucidation of cellular signaling pathways, and the like.

Biological responses, particularly responses in primary human cells, can display significant variability from day to day and from donor to donor. One important aspect of the present invention is that, while the levels of determined parameters can vary substantially between assays, combinatorial responses involving multiple pathways are less variable. Thus, the process of normalization used to produce a biomap provides cellular activity profiles that are robust and reproducible.

In contrast, Friend et al., which primarily rely on a simplified cell model provided by yeast cells, find that:

The methods of the present invention include: (i) obtaining or providing response profiles for the biological response (or responses) of interest; (ii) defining sets of co-regulated cellular constituents (i.e., genesets) in the response profiles; and (iii) identifying common response motifs among the defined sets of co-regulated cellular constituents which are associated with particular biological responses such as drug effectiveness or toxicity. The common response motifs thereby identified comprise the consensus profiles of the invention. In preferred embodiments, the methods of the invention further include the step (iv) of "projecting" the original response profiles onto the genesets identified in step (ii) above. Simplified, reduced-dimension response profiles are thereby produced which are more simply and robustly related to biological properties such as drug effectiveness and toxicity.

In contrast, Applicants' invention does not define sets of co-regulated cellular constituents (step ii above) or step (iii) above, defining common response motifs among the sets. Friend et al. require these steps because the reference is directed to deriving a consensus profile from a desired or "ideal" agent (sifting through all of the transcripts to find a set that reproducibly co-regulates). In the case of Friend et al., for every "ideal" agent that they would like to compare against, they will derive a different consensus profile. Thus, every different consensus profile will contain a different gene set. In the present invention, the same cellular constituents (i.e. readout parameters) are determined for all test agents, and cellular constituents that are not altered by the "ideal" agent are just as important as those that are up or down-regulated. Indeed, Friend et al. emphasizes the use of co-varying cellular constituents, whereas the present invention selects cellular constituents that preferably do not co-vary, and are independent.

The secondary reference fails to remedy the deficiencies of the primary reference. While Chung et al. teach methods of cell culture, the reference fails to teach the activation of multiple pathways, the testing of agents in such activated cells, and the use of multiple parameters to determine pathway activation.

Applicants respectfully submit that the cited art fails to teach or suggest the presently claimed invention.

With respect to the claims of Group II (Claim 19), Appellants reiterate the arguments made with respect to Group I, and further submit that Friend *et al.* nowhere teaches primary cells and, in every case, instead teaches the use of cell *lines*, including yeast cell lines, in carrying out its methods. One of skill in the art understands that a primary cell is not a cell line. Cells that are cultured directly from a subject are known as *primary cells*. Primary cell cultures typically have a limited lifespan, and can be sensitive to factors and agents that do not affect established cell lines. After a certain number of population doublings cells undergo the process of senescence and stop dividing. An established or immortalised *cell line* has acquired the ability, either through random mutation or deliberate modification, to proliferate indefinitely. There are numerous well established cell lines representative of particular cell types.

Appellants note column 45, section 5.5.2 of Friend *et al.*, by way of example, which recommends the use of the Jurkat T *cell line* as a model cell on which to conduct transfection or transduction techniques in order to generate cell lines with controllable perturbed expression for studying immunosuppressive drug candidate effects. Friend *et al.* states:

A particular example of the use of this method is the search for drugs that target the src-family protein tyrosine kinase, lck, a key component of the T cell receptor activation pathway (Anderson *et al.*, 1994, *Adv. Immunol.* 56:171-178). Inhibitors of this enzyme are of interest as potential immunosuppressive drugs (Hanke J H, 1996, *J. Biol Chem* 271(2):695-701). A specific mutant of the Jurkat T cell line (JCaM1) is available that does not express lck kinase (Straus *et al.*, 1992, *Cell* 70:585-593). Therefore, introduction of the lck gene into JCaM 1 by transfection or transduction permits specific perturbation of pathways of T cell activation regulated by the lck kinase. The efficiency of transfection or transduction, and thus the level of perturbation, is dose related. The method is generally useful for providing perturbations of gene expression or protein abundances in cells not normally expressing the genes to be perturbed.

Thus, where Friend *et al.* identifies as a goal the study of T cell activation, Friend *et al.* suggests the use of Jurkat T cells, an immortalized T cell *line*, instead of primary cells. Further, Friend *et al.* teaches the use of yeast cells to study the effects of drug candidates (see Sections 5.3.1, 5.3.5 and the reference generally). The Chung *et al.* reference teaches only liver epithelial cell *lines*. Accordingly, the cited references provide no guidance to the ordinarily skilled artisan to use of primary cells for studying the mechanism of agents on cell pathways; indeed, the references teach to the contrary.

With respect to the claims of Group III (Claim 20), Appellants reiterate the arguments made with respect to Group I, and further submit the purpose of the Friend *et al.* methods, as described in the Invention Summary, is:

for determining a "consensus" profile for a biological response, such as the response of an organism to a group or family of drugs and/or drug candidates. The consensus profile obtained by the methods of this invention represents an ideal, desired activity profile across some standard measurement set such as the cellular constituents of a cell or model organism, or of an organism destined for treatment, e.g., by drug therapy. As such, the consensus profiles of this invention indicate those elements or patterns in a biological profile which the individual compounds have in common. Preferably, such elements or patterns are associated with a particular biological effect--most preferably a particular, desired, therapeutic effect, or "ideal" effect. Accordingly, the present invention also provides methods for obtaining a response profile for a particular compound, such as for a particular drug or drug candidate, and for comparing the response profile of the particular compound to the consensus profile to determine the extent to which the particular compound exhibits a particular, i.e., "ideal," effect as opposed to "non-ideal" or toxic effects.

This passage makes clear that Friend *et al.* does not teach the use of at least one activator of a pathway active in a cell culture. Friend *et al.* methods employ measurements that simply define sets of co-regulated cellular constituents, and define common response motifs among the sets in response to a test drug. The methods of Friend *et al.* require these steps, because the reference is directed to deriving a consensus profile from a desired or "ideal" agent (sifting through all of the transcripts to find a set that reproducibly co-regulates).

Put simply, whereas Friend *et al.* collects gene expression data by exposing cells to a given drug and determining clusters of genes which covary in response to that drug, the present methods instead contacts an agent with cells in culture that are stimulated in multiple pathways by the addition of at least two factors. Appellants respectfully submit that there is no teaching by Friend *et al.* in combination with Chung *et al.* that would inform one of skill in the art to perform such analysis in the presence of at least two factors acting on the cell, whether the factors are activators or suppressors.

With respect to the claims of Group IV (Claim 21), Appellants reiterate the arguments made with respect to Group I, and further submit, as cited above, the purpose of Friend *et al.* methods as described in the Invention Summary is:

for determining a "consensus" profile for a biological response, such as the response of an organism to a group or family of drugs and/or drug candidates. The consensus profile obtained by the methods of this invention represents an ideal, desired activity profile across some standard measurement set such as the cellular constituents of a cell or model organism, or of an organism destined for treatment, e.g., by drug therapy. As such, the consensus profiles of this invention indicate those elements or patterns in a biological profile which the individual compounds have in common. Preferably, such elements or patterns are associated with a particular biological effect--most preferably a particular, desired, therapeutic effect, or "ideal" effect. Accordingly, the present invention also provides methods for obtaining a response profile for a particular compound, such as for a particular drug or drug candidate, and for comparing the response profile of the particular compound to the

consensus profile to determine the extent to which the particular compound exhibits a particular, i.e., "ideal," effect as opposed to "non-ideal" or toxic effects.

This passage makes clear that Friend *et al.* does not teach the use of at least one inhibitor of a pathway active in a cell culture. The methods of Friend *et al.* employ measurements simply to define sets of co-regulated cellular constituents, and to define common response motifs among the sets in response to a test drug. The methods of Friend *et al.* require these steps, because the reference is directed to deriving a consensus profile from a desired or "ideal" agent (sifting through all of the transcripts to find a set that reproducibly co-regulates).

Put simply, whereas Friend *et al.* collects gene expression data by exposing cells to a given drug and determining clusters of genes which covary in response to that drug. In contrast, Appellants' methods employ a step in which a test agent is contacted with cells in culture that are stimulated in multiple pathways by the addition of at least two other factors. Appellants respectfully submit that there is no teaching by Friend *et al.* in combination with Chung *et al.* that would inform one of skill in the art to perform such analysis in the presence of at least two factors acting on the cell, whether the factors are activators or suppressors, such that the resulting dataset is indicative of the pathways that are active in the culture.

In view of the discussion above, the Appellants submit that Friend *et al.* in view of Chung *et al.* fails to teach or suggest the claims of any of Groups I through IV and respectfully request reversal of this rejection.

IV. Claims 17 and 19-22 are not made obvious by the combination of Friend *et al.*, U.S. Patent no. 6,801,859 in view of Rice *et al.* (1996) Analytical Biochemistry 241:254-259.

Appellants respectfully submit that the presently claimed invention is not taught or suggested by the cited combination of Friend *et al.* in view of Rice *et al.* The arguments and grouping of claims with respect to Friend *et al* are as argued above.

Appellants respectfully submit that the secondary reference, Rice *et al.*, fails to remedy the deficiencies of the primary reference. Rice *et al.* teach methods with, at most, only superficial similarity to those of the invention. The prior art methods culture HUVECs, as shown in Figure 2A-C, in the presence of a factor, or of pooled conditioned medium. A single parameter (E-selectin) is measured.

Rice *et al.* then describe a test for inhibitors of HUVEC stimulated with the single factor, IL-1 $\beta$  at 0.1 ng/ml, with a single output parameter, E-selectin expression.

The methods taught by Rice *et al.* differ from those of the present invention in important features of the invention. Rice *et al.* methods do **not**:

- Contact cells activated in multiple pathways with a test agent. In contrast to the methods of the invention, Rice *et al.* methods only involve the addition of IL-1 $\beta$  to cells for screening; the methods do not stimulate multiple pathways in the test cells.
- Compare results against activated cultures in the absence of the test agent.
- Involve analysis of multiple parameters. Rice *et al.* methods measure only a single parameter.

The "pooled conditioned medium" utilized by Rice *et al.* is not equivalent to adding multiple factors *and* a test agent to a culture. Applicants respectfully submit that the Rice *et al.* reference does not teach the addition of a test agent to cell cultures in which pooled conditioned medium has been added. A test agent is added to the cells stimulated with a single factor – the reference notes that the LPS-conditioned plasma was eliminated as a potential agonist for the screen because of the difficulties obtaining inhibition.

An important point is that the method of Rice *et al.* does not and cannot classify an agent according to pathways that are activated. Because the readout of the Rice *et al.* method is insufficiently informative, one cannot determine the affect on specific pathways.

In the data provided by Appellants, (Table 1 excerpt above) it can be seen that, if one were to judge a compound solely by its effect on the single parameter, E-selectin, that the mechanism of action could not be distinguished between controls, antioxidant, corticosteroids, or a tyrosine kinase inhibitor. It is the recognition by Applicants that multiple factors activating multiple pathways and measuring multiple parameters are required for meaningful analysis of pathway action that provides for the unexpected benefits of the presently claimed methods.

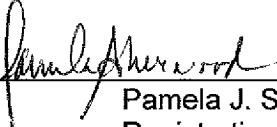
In view of the discussion above, the Appellants submit that Friend *et al.* in view of Rice *et al.* fails to teach or suggest the claims of any of Groups I through IV and respectfully request reversal of this rejection.

**RELIEF REQUESTED**

The Appellants respectfully request that the rejection of Claims 17 and 22 under 35 U.S.C. § 102(b) and the rejection of Claims 17 and 19-22 under 35 U.S.C. §103(a) be reversed; and that the application be remanded to the Examiner with instructions to issue a Notice of Allowance.

Respectfully submitted,

Date: March 2, 2009

By: 

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CLAIMS APPENDIX

17. (previously presented) A method for analyzing a candidate compound for a biological activity of interest, the method including:

contacting a test mammalian cell culture with the compound, wherein the culture includes a plurality of factors wherein a plurality of signaling pathways are induced by the presence of the factors;

measuring at least two parameters associated with the plurality of pathways and comparing the measurement of the at least two parameters with the measurement from a control cell culture lacking the compound, and

recording the measurements of the test cell culture and the control cell culture to produce a biological dataset profile, wherein the biological dataset profile is indicative of the pathways that are active in the cell culture.

19. (previously presented) The method of Claim 17, wherein the cells are primary cells.

20. (previously presented) The method of Claim 17, wherein the test cell culture includes at least one activator of a pathway active in the cell culture.

21. (previously presented) The method of Claim 17, wherein the test cell culture includes at least one inhibitor of a pathway active in the cell culture.

22. (previously presented) The method of Claim 17, further including the step of compiling a plurality of the biological dataset profiles in a database.

**EVIDENCE APPENDIX**

No evidence that qualifies under this heading has been submitted during the prosecution of this application, and as such it is left blank.

**RELATED PROCEEDINGS APPENDIX**

As stated in the *Related Appeals and Interferences* section above, there are no other appeals or interferences known to Appellants, the undersigned Appellants' representative, or the assignee to whom the inventors assigned their rights in the instant case, which would directly affect or be directly affected by, or have a bearing on the Board's decision in the instant appeal. As such this section is left blank.